better elucidate this, our group is conducting a prospective study that evaluates the T cell receptor (TcR) Vβ repertoire from patients who are undergoing either matched related (MRD) or unrelated (URD) donor allogeneic hematopoietic stem cell transplantation (HSCT) for treatment of hematologic malignancies. These patients received conditioning therapy with a regimen combining rabbit anti-thymocyte globulin (Thymoglobulin®, Genzyme, Cambridge, MA) and 450 cGy total body irradiation. We used a novel two-tier analysis of TcR Vβ mRNA expression in order to identify GVHD and/or GVT-associated T cell clones: i) normalization of the TcR Vβ mRNA to CD3 mRNA rather than to housekeeping genes using real-time RT-PCR which increases sensitivity of the assay for detecting minimal circulating T cell clones of interest that are activated; ii) comparative analysis of the recipients' TcR Vβ over those of the donors' at the time of GVHD or GVT effects up to 1 year post-HSCT. We then performed spectratyping analysis to confirm clonality of the relevant T cell clones. We have been able to identify T cell populations in the HSCT recipients at 90 days to 1 year following transplant, by demonstrating that distinct TcR Vβ transcripts were significantly increased relative to the steady state expression in the donor, in patients without GVHD or relapse vs. patients with GVHD. In the three GVHD-free, relapse-free patients, we found an increased expression of Vβ 9 in all patients as well as an increased expression of Vβ 16 and Vβ5/Vβ 24 in two and one patient, respectively. In 6 patients with GVHD, we observed increased expressions of Vβ4 and Vβ11 in four patients and increased Vβ23 in three patients. Spectratyping analyses of each of these TcR Vβ families showed distinct skewed, mono- or oligoclonal populations, in patients with GVHD or GVHD- and relapse-free states. The identification of these unique T cell clones in patients who have developed GVHD or GVHD-free responses after allogeneic HSCT suggests that they may be driven by antigen stimulation uniquely associated with GVHD or GVT. Identification of GVHD-associated T cell clones may offer aan opportunity for targeted therapy of GVHD rather than using conventional immunosuppressive drugs.

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SIROLIMUS, TACROLIMUS, AND RABBIT ATG (R-ATG) AS GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN PATIENTS UNDERGOING UNRELATED DONOR PERIPHERAL BLOOD HEMATOPOIETIC CELL TRANSPLANT (HCT): EXTENDED FOLLOW UP AND UPDATED ANALYSIS

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The combined use of Sirolimus (SIR) and Tacrolimus (FK) +/− low-dose methotrexate (MTX) recently showed a promising result in preventing acute GVHD after unrelated donor HCT (Antin et al. Blood 2003) although a significant number of patients still experienced chronic GVHD. In an attempt to further improve the outcome, we have evaluated a novel combination of Tacrolimus, Sirolimus and r-ATG (4.5 mg/kg) +/− Methotrexate (for patients with a mismatched donor). We have previously presented the early results of this combination. In this report we extended the follow-up period and updated analysis focused on 49 patients who received PBSCT (See Table 1 for patient demographics).

Results: Engraftment rate was 91.8% (n = 45) with the median neutrophil engaftment at 15 days (range: 10-39). Seventeen patients (38% of 45 engrafted) developed grade II-IV acute GVHD (grade III = 3, IV = 0). Chronic GVHD developed in 23 of 38 evaluable patients (60%, limited n = 4, extensive n = 19). We observed TTP/HUS in 10 patients (20%) and one case of VOD. Eighteen (42%) of 43 patients at risk (Recipient +ve or Donor +ve) developed CMV reactivation, while 10 patients developed EBV reactivation (19.6%). After a median follow up of 24 months (range: 6-48) for 33 surviving patients, the **1-year OS, DFS, and relapse rate** were **75.4%** (95%CI: 64.8-83.2), **73.4%** (63.0-81.3), and **8.2%** (3.2-21.0), respectively. Non-relapse mortality (NRM) was 16.3% (8.7-30.8) at 100 day and 18.4% (10.2-33.2) at 1 year. There were no significant differences in the outcomes according to conditioning regimens, although there was a trend for lower NRM with Flu/Mel (9.7% vs. 27.8% at 1 year, p = 0.06).

In summary the combination of FK/SIR with r-ATG +/− MTX may improve the GVHD and survival outcome in this high-risk population. However, we observed graft failure in 4 patients, which appeared to be a higher rate than our historic data of approximately 2% in the setting of unrelated donor HCT.

Table 1. Patient Demographics

Variable	All Patients N=49 (100%)
Patient Gender	
Female	28 (57.1)
Male	21 (42.9)
Median Age at transplant	57 (19-71)
Disease / Disease status	
ALL/CR1 and CR2	9
ALL/Induction failure	1
AML/CR1 and CR2	12
AML/Relapse and Induction failure	7
CML/CP1 and CP2	2
CML/ AP and BC	2
NHL	5
CLL	1
MDS	8
MPN	2
Conditioning Regimen	
Reduced Intensity Fludarabine/ Melphalan	31
FTBI/Cytoxan	7
FTBI/VP-1611	11
HLA Match	
10 out of 10	26
others	23

ALL; Acute Lymphoblastic Leukemia, CR; Complete Remission, CP; Chronic Phase, AP;Accelrated Phase, BC;Blast Crisis, NHL; Non-Hodgkin Lymphoma, CLL;Chronic lymphocytic leukemia, MDS;Myelodysplastic Syndromes, MPN; Myeloproliferative Neoplasm

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NRF2 REGULATES ALLOREACTIVE T CELL FUNCTION DURING GVHD

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The activation and regulation of donor alloreactive T cells is critical for the development of graft-versus-host disease (GVHD) as well as the regulation of graft-versus-tumor (GVT) activity. To better understand transcriptional orchestration of alloreactive T cells, we employed Finding Informative Regulatory Elements (FIRE) approach, recently published by Elemento et al, to investigate gene expression data in donor T cells in murine syngeneic (syn-) and allogeneic bone marrow transplant (allo-BMT) models. We discovered the presence and absence of the motif CCGGAAG, recognized by the transcription factor Nuclear Factor Erythroid 2-Like Factor 2 (Nrf-2), correlated to a set of genes that were slightly upregulated in syn- and downregulated in allo-donor T cells. We hypothesized that Nrf-2 serves as a master regulator of genes in alloreactive donor T cells involved in the GVHD setting.

We first examined the level of expression of Nrf-2 in donor T cells in murine syn- and allo-BMT models. We found that the expression of Nrf-2 was significantly reduced only in the regulatory T cell population (CD4+CD25+Foxp3+) in allo-BMT ($p = 0.0004$). We next questioned the significance of Nrf-2 in alloreactive T cells. Adoptive transfer of Nrf2-/- donor T cells leads to less GVHD morbidity, as characterized by weight loss ($p < 0.0001$) and better survival ($p = 0.0030$), compared to Nrf-2+/+ donor T cells. Interestingly, loss of functional Nrf-2 in donor T cells did not perturb their GVT activity at the studied dose (A20, 0.25x106 per host). We demonstrated that CFSE labeled Nrf2-/- donor T cells have intact proliferation and activation (as determined by the upregulation of CD25) upon stimulation by alloantigen. Lastly, we investigated the trafficking ability of Nrf2-/- donor T cells to gut-associated lymphoid tissues. Although not statistically significant, there were less Nrf2-/- donor T cells infiltrating the mesenteric lymph nodes (MLNs) compared to Nrf2+/+